

BEHAVIOR OF DAPHNIA IN POLARIZED LIGHT¹

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Three models have been proposed to account for the apparent ability of animals to perceive the plane of vibration of polarized light. Two of the proposed models are intra-ocular, the third is extra-ocular. The three models are: (1) a radial array of dichroic filters (rhabdomeres) with their fast axes tangential to the radii of the array; (2) the Brewster-Fresnel models in which one or more refractions and reflections at corneal or lens surfaces serve to diminish preferentially the intensity of light polarized parallel to the plane of incidence; (3) the reflected brightness pattern in which the intensity of light reflected and scattered from the environment is least parallel to the polarization plane and greatest perpendicular to the polarization plane.

Two Brewster-Fresnel models have been proposed. The Brewster-Fresnel reflection model relying upon a single refraction was proposed by Stephens, Finger-man and Brown (1953) for the *Drosophila* eye. The Brewster-Fresnel reflection model relying upon internal reflection from a lenticular surface was proposed by Baylor and Smith (1953) for *Daphnia*. That daphnids utilize an intra-ocular analyzer in clear water was established by Baylor and Smith (1960) using half-wave plates to distinguish between intra-ocular and extra-ocular polarization analyzers. These experiments corroborated their earlier findings (Baylor and Smith, 1953) as well as those of Waterman (1960). To test the Brewster-Fresnel internal reflection model, Baylor and Hazen (1962) conducted optical analyses of the lenses of *Daphnia pulex* (de Geer), including a microphotometric study of polarized light transmitted by the lenses. Their results are in agreement with the Brewster-Fresnel internal reflection model. The present paper examines the consequences of this model on the behavior of daphnids under polarized light.

We assume that in its response to polarized light, the daphnid moves so that the rhabdomeres of the forward ommatidia receive maximum light intensity. If this assumption is true, then the addition of light to the lateral ommatidia should disrupt the precision of the daphnid response to the polarization plane. The degree of disruption should be proportional to the amount of light added to the lateral ommatidia.

For polarization detection, the intensity of light at the rhabdomeres is maximum when the polarization plane of the incident light is perpendicular to the long axis of the cone. The forward and lateral ommatidia are perpendicular to each other in a horizontal plane, and therefore present mutually perpendicular planes of incidence to a vertical beam of light, as in Figure 1. The Fresnel equations require that whenever the forward-directed ommatidium has a maximum intensity at the

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rhabdomeres, then the laterally directed ommatidium has a minimum intensity at its rhabdomeres; the ratio of the intensity is approximately 8:1. Experimentally changing this ratio by directing horizontal light beams at the lateral ommatidia of a population of daphnids already orienting to a vertical beam of polarized light should produce an orientation in which the number of animals directed to the stimulus of the lateral beam is the same as the number directed by the vertical beam.

We report here three sets of experiments. The first, with nonpolarized light, shows how a population of *Daphnia* oriented to two horizontally opposed light beams (AB in Fig. 2) changes orientation upon the addition of a second pair of horizontally opposed light beams (CD in Fig. 2) perpendicular to the first. This experiment tests the validity of the primary assumption on which the Brewster-Fresnel internal reflection model rests, *i.e.*, that positive phototaxis is guided by

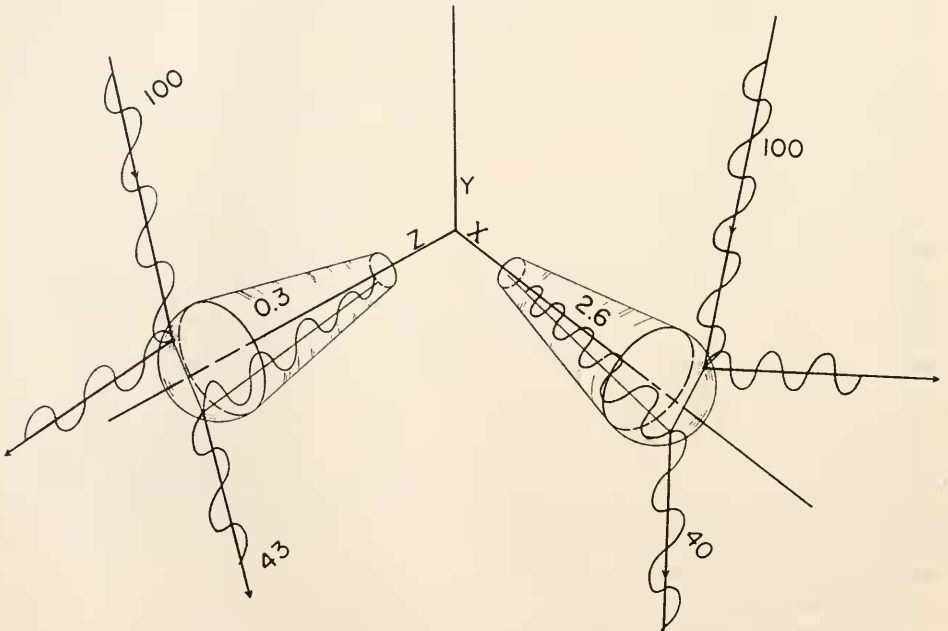


FIGURE 1. Three-dimensional diagram of the Brewster-Fresnel internal reflection model, showing two cone lenses at right angles. Polarized light is incident from above with an incident value of 100. The plane of polarization is parallel to the YZ plane. Numbers represent intensities at various parts of the light paths.

maximum intensity reception in the forward ommatidia. The second set of experiments shows how the orientation to a vertical polarized beam is altered by a pair of horizontally opposed beams parallel to the plane of polarization; in this experiment the lateral beam illuminates the lateral ommatidia of those animals responding to the plane of polarization. The third set of experiments shows how daphnids which appear to be primarily photonegative nevertheless have a secondary, weaker positive phototaxis which operates at right angles to the primary and vigorously negative phototaxis.

PROCEDURE

Experimental animals were from a laboratory culture of *Daphnia pulex* (de Geer) grown under constant light and fed a mixture of algae and yeast daily. Approximately a hundred of these animals were placed in filtered water in a lucite tank one foot on each side and shielded from stray light in a darkened room. A projection lamp with lenses and a polarizer hung four feet above the tank and provided a linearly polarized light beam. A black shield prevented light from shining on the sides of the tank and being reflected from them. The irradiance of this beam on the tank was approximately 100 foot lamberts. Two opposed, matched projection lamps were placed so that their beams were parallel to the plane of polarization; a second pair was placed perpendicular to the first (Fig. 2). The brightness of these lamps could be varied with neutral density filters or by a variable

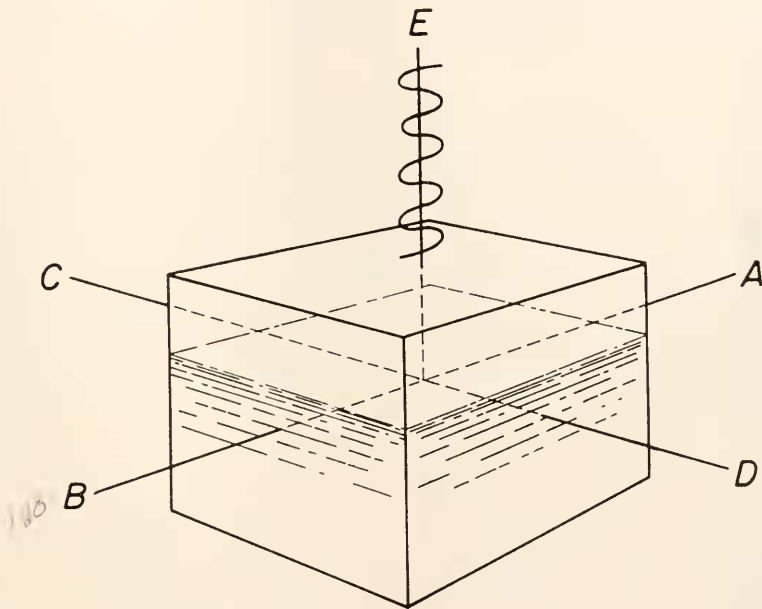


FIGURE 2. Diagram of test tank and its illumination. AB and CD are pairs of horizontally opposed light beams; E is a vertical beam polarized in ABE plane. CD is perpendicular to AB.

transformer. Light intensities were measured with a model 501M Photovolt photometer.

To record the orientation of the swimming animals during a test, a time exposure photograph of three seconds was made. The path of each moving daphnid in the field was represented by a line on the photograph. The directions of these lines were measured with a protractor, and the measurements were grouped into twelve intervals of 15° each. The midpoints of these intervals were $0^\circ = (180^\circ)$, line AB in Fig. 2), 15° , 30° , 45° , . . . 165° . The 0° azimuth was parallel to the plane of polarization of the overhead light beam (E in Fig. 2) and also to one pair of hori-

zontally opposed light beams (AB in Fig. 2). In the experiment without the overhead polarized light two pairs of horizontally opposed light beams were employed, one at the 0° azimuth and the other at the 90° azimuth. In another set of experiments in which only side lights were used, the single pair of opposed horizontal beams was parallel to the 90° azimuth.

For convenience in discussing and manipulating the data we may calculate an

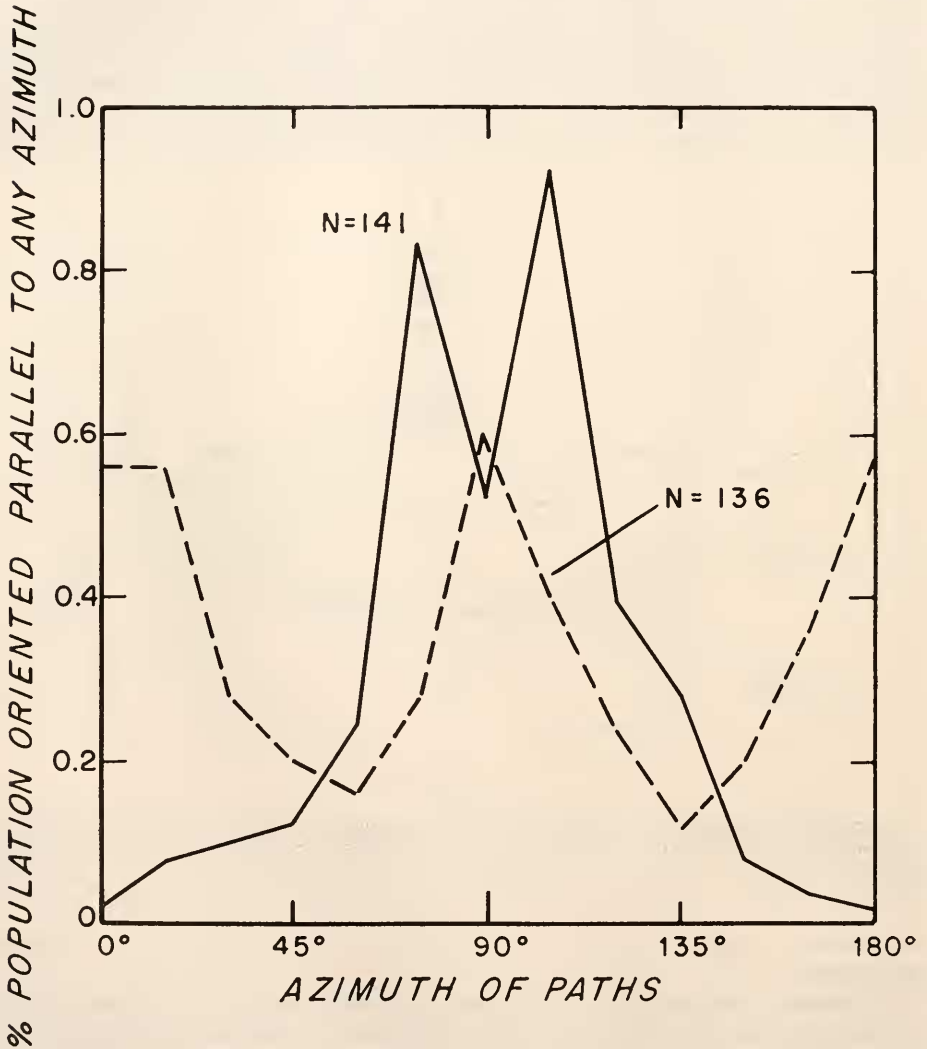


FIGURE 3. The per cent of a population of *Daphnia* oriented at various azimuths relative to horizontal, nonpolarized light beams. Solid line represents the data from a single pair of opposed light beams parallel to the 90° azimuth. Dotted line represents data from two pairs of opposed beams perpendicular to each other, one pair parallel to the 0° azimuth, the other pair parallel to the 90° azimuth.

index of the angular orientation relative to any given azimuth from the following relation:

$$\frac{P_{\theta} - P_{\theta+90^{\circ}}}{P_{\theta} + P_{\theta+90^{\circ}}} = \text{I. O.}, \text{ the index of angular orientation:}$$

where P_{θ} is the ratio between paths parallel to the azimuth heading θ and all paths, and $P_{\theta+90^{\circ}}$ is the ratio of paths parallel to the azimuth heading $\theta + 90^{\circ}$ and all paths. The measurements of orientation were grouped by 15° intervals as stated above. In presenting the data, running averages of three groups are used and thus orientation for a given angle includes all organisms oriented within 22.5° of that angle. Since each ommatidium subtends an angle of 50° , greater precision of orientation implies some integration of receptor information. The index of orientation can vary from plus one to minus one with zero being an indication of equal amounts of behavior in both directions, which would include random behavior.

RESULTS

The solid line of Figure 3 shows the orientation of 141 *Daphnia* to a single pair of horizontally opposed light beams. The response, with maxima at 75° and 105° , is approximately parallel to the light beams which are directed along the 90° azimuth. The average index of orientation at these two peaks is 0.87. The lower index of orientation at 90° is unexplained but has been reproduced in several experiments. The dotted line shows the orientation of daphnids to four matched lights 90° apart in the horizontal plane. The responses to the two perpendicular pairs of beams are parallel to the beams and are nearly equal with an index of orientation of -0.09 .

Results of the experiment with one pair of opposed beams, described by the solid line of Figure 3, appear to support the assumption that daphnids possess a positive phototaxis and orient by maintaining maximum light intensity in the forward ommatidia.

The experiment with two pairs of opposed beams shows that when the front and lateral ommatidia are equally illuminated, the population of daphnids has equal numbers of animals orienting to each pair of opposing beams and thereby further supports the assumption that positive phototaxis is guided by maintenance of maximum intensity in the forward ommatidia. The data show that the daphnid compound eye does not act simply as a receptor consisting of a large number of parts, each obeying a cosine² law, where the intensity at the receptor will be equal to some constant times the cosine² of the angle of incidence. If the daphnid eye did obey the cosine² law, the 4-beam experiment would produce random results. That the daphnid eye could not obey the cosine² law is also clear because each ommatidium subtends an angle of approximately 50° , thereby limiting the angle through which each ommatidium can receive light directly.

We know from the 4-beam experiment what the response of a population of daphnids is when the front and lateral ommatidia are equally illuminated. What, then, will be the effect of an overhead polarized beam in combination with one pair of horizontally opposed lateral beams which can be varied in intensity to produce various ratios of overhead polarized light intensity to lateral nonpolarized light intensity?

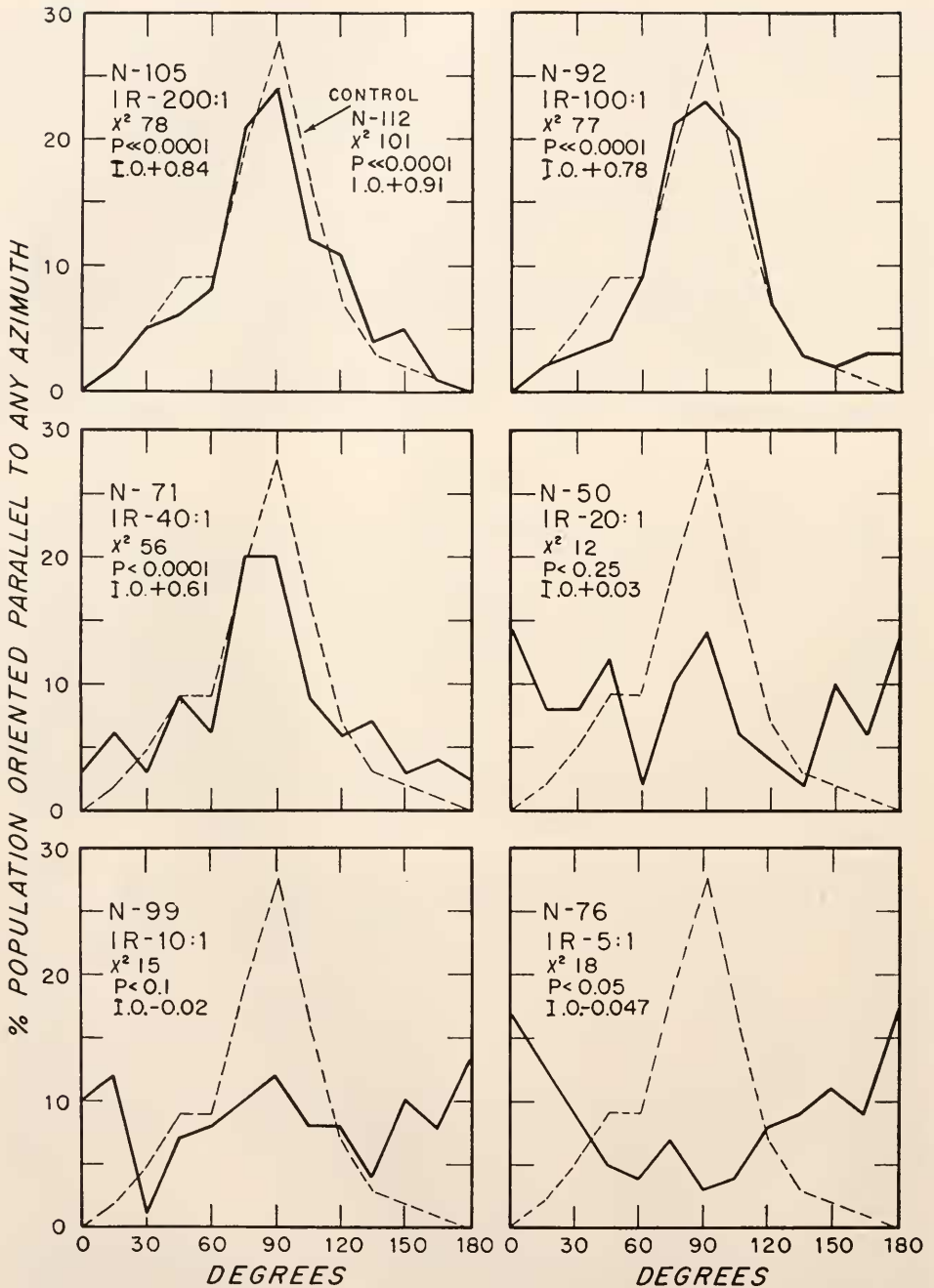


FIGURE 4. Orientation of *Daphnia* at various azimuths relative to the plane of polarization (0-180°) at six different ratios of intensities of vertical to horizontal light beams. The vertical beam is polarized and at a constant intensity. The pair of horizontal, opposed beams is parallel

TABLE I
A summary of data

Intensity ratio	Number of paths measured	Chi ²	P	Index of orientation
Control	112	101	.0001	+0.91
200:1	105	78	.0001	+0.84
100:1	92	77	.0001	+0.78
40:1	71	56	.0001	+0.61
20:1	50	12	0.25	+0.03
10:1	99	15	0.10	-0.02
5:1	76	18	0.05	-0.047
2 Beams	136			-0.87
4 Beams	176			0.09

Figure 4 shows six graphs of the response of *Daphnia* to different intensities of lateral light, the overhead polarized beam remaining constant. The ratios of the overhead polarized intensities to side nonpolarized intensities (IR in Fig. 4) were chosen so that some were higher than the balance point ratio predicted on a theoretical optical basis, and some were lower. Each graph is plotted with the data from a control experiment, shown as a dotted line, in which only the vertical polarized beam is present. The data on the graphs are summarized in Table I. In the control, Chi square for the null hypothesis that the direction of swimming is random is 101, giving a probability much less than 1 in 10,000 that the behavior is random. The Chi square tests for the different intensities of lateral light are included in Figure 4. An examination of the graphs in sequence from that showing an intensity ratio (IR) of 200:1 to that of 5:1 shows a gradual change in orientation. At 200:1 and 100:1 the effect of lateral light intensity is minimal. At 20:1 the Chi square test gives a probability of the orientation being different from random orientation of only 0.25, showing that at this ratio the intensity apparent to the animal is nearly the same parallel and perpendicular to the plane of polarization. At the ratio of 5:1 the taxic response is oriented more toward the lateral light than it is to the stimulation offered by the polarized beam.

The calculated ratio of overhead polarized intensity to side nonpolarized intensities produced at the rhabdomeres of two perpendicular ommatidia by a vertical beam of polarized light is 8:1, as shown in Figure 1. The index of orientation with this ratio is +0.91. Throughout the range of intensity ratios of vertical to horizontal illumination there is a graded response. This graded response is best seen in Figure 5 where the index of orientation is plotted against the intensity ratios. Because the magnitude of the electroretinogram is proportional to the logarithm of the intensity of the stimulating light (Hartline, 1930), the intensity ratios of Figure 5 were plotted as logarithms. The data points seem to fall in a straight line

to the plane of polarized light and varies in intensity. Abbreviations: N is number of animals used; IR is the intensity ratio of overhead polarized to lateral nonpolarized light beams; χ^2 is Chi square value; P is the probability that the orientation is random; I.O. is index of orientation.

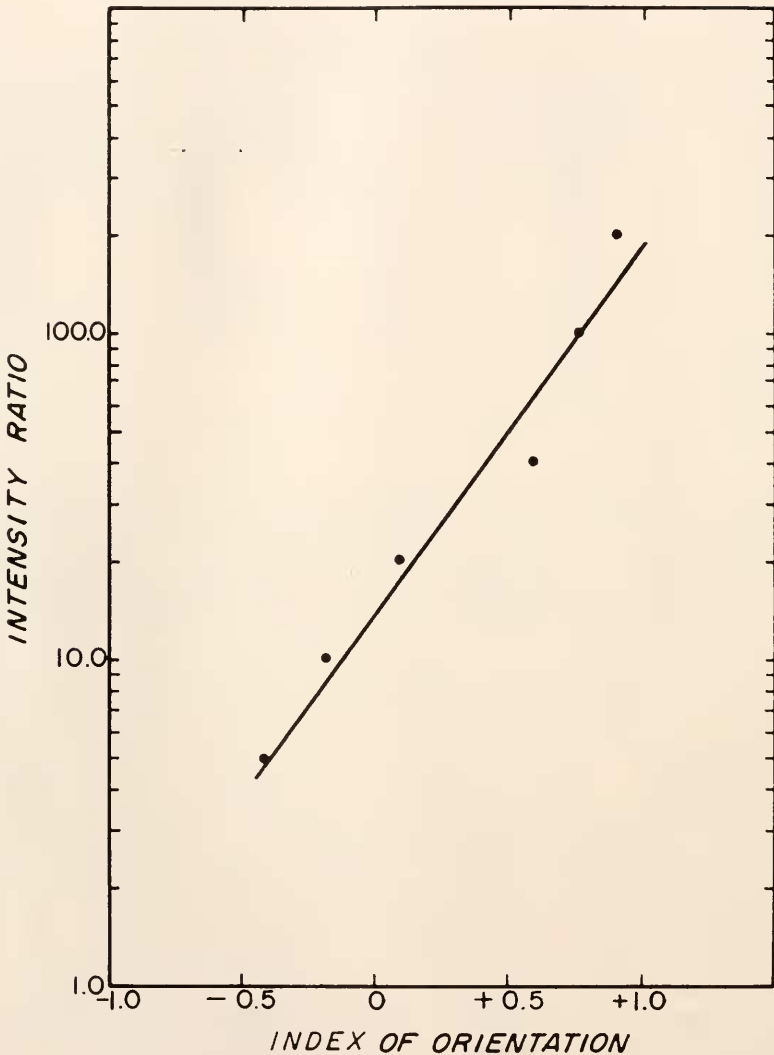


FIGURE 5. The degree of orientation at various intensity ratios of vertical to horizontal light beams. The scale of the intensity ratios is logarithmic.

which was drawn by eye. We interpret the intercept of this line with the zero value of the abscissa to be the ratio at which the intensities in the lateral and forward ommatidia are equal. From the graph this value is approximately 15:1. By theory this ratio should be approximately 8:1 as calculated for Figure 1. In view of the uncertainty of the assumptions made concerning the index of refraction and the shape of the lenses we think this discrepancy is small.

The data do not, of course, distinguish between the Brewster-Fresnel internal reflection model and all other models. A single refraction with the light path direct to visual pigments (Stephens *et al.*, 1953) would produce maximum intensity

in lateral ommatidia when daphnids orient perpendicular to the polarization plane. On the other hand, a refraction followed by a reflection would produce maximum intensity in the forward ommatidia when daphnids are similarly oriented. If responses to polarized light are based on the same physiological mechanisms as positive phototaxis (and the 4-beam experiment strongly supports this hypothesis), then the Brewster-Fresnel refraction model of Stephens *et al.* is ruled out for daphnids, but the Brewster-Fresnel internal reflection model is not ruled out.

In a further attempt to test the assumption that the orientation to polarized light is essentially a phototactic response in which the forward ommatidia are kept bright, we studied the behavior of daphnids made photonegative by drugs or by ultraviolet light. In these animals we expected to find orientation parallel to the plane of polarization rather than perpendicular, but this expected orientation did not occur. The failure of photonegative daphnids to orient parallel to the polarization plane has constituted a major criticism of the Brewster-Fresnel reflection model (personal communication from Colin Pittendrigh and from Rudolph Jander). Clearly, we must resolve this apparent paradox or abandon the model altogether.

The paradox may be resolved if two separate and distinct phototaxes are involved. The primary and obvious phototaxis response of pilocarpine-treated daphnids to an intense parallel light beam is a vigorous negative phototaxis. The secondary phototactic response of these animals is a weakly positive phototaxis to any dim light beam perpendicular to the intense beam. This paradoxical behavior of daphnids is consistent with their possession of two separate photoreceptors having quite different functions (Baylor and Smith, 1957): the compound eye is sensitive to polarized light, whereas the naupliar eye appears to control the sign of phototaxis and geotaxis in response to a number of chemical and physical factors of the environment. The behavior of daphnids in the natural habitat shows an obvious adaptive value for these two distinct and separate phototaxes executed approximately at right angles to each other. A negative and a positive phototaxis to the sun are presumably useful for guiding vertical migration, and at the same time a positive phototaxis for light scattered from phytoplankton or other food particles permits food-finding during the day when daphnids are photonegative. In daphnids made vigorously photonegative by treatment with 10^{-6} M pilocarpine, the change in behavior produced by adding a horizontal beam to the vertical polarized beam was compared with the same experiments in which the animals were untreated and photopositive. Results of preliminary experiments show no significant difference between vigorously photonegative drug-treated daphnids and untreated photopositive daphnids. The data points from these experiments fall on the curve of Figure 5.

We are hopeful of finding another drug which will reverse the secondarily positive phototaxis normally associated with finding food. When this is done we may then anticipate that such animals treated in this way will orient parallel to the polarization plane of an overhead light.

SUMMARY

1. Daphnids illuminated by a single vertical beam of polarized light swam approximately perpendicular to the polarization plane.

2. Daphnids illuminated by a single pair of opposed horizontal beams of light oriented toward the brighter light of the pair.

3. Daphnids illuminated by two pairs of opposed horizontal beams set at right angles to each other swam in the beam of the brighter pair of light beams.

4. Daphnids illuminated simultaneously by three beams (one polarized and coming from overhead, the other two nonpolarized and horizontally opposed, parallel to the polarization plane of the overhead light) responded to the overhead polarized light when its intensity was greater than 20 times that of the horizontal beams. When the intensity of the overhead beam of polarized light was less than 20 times that of the horizontal beams, the daphnids responded to the horizontal opposed beams instead of the polarized beam from overhead.

5. The changes in behavior induced by various intensity combinations of overhead and horizontal light beams were in good agreement with the changes predicted from daphnid eye structure.

6. Daphnids exhibiting drug-induced negative phototaxis were shown to possess simultaneously a secondary weak positive phototaxis always executed at right angles to the negative phototaxis. This weak positive phototaxis at right angles to the negative phototaxis is proposed to account for photonegative daphnids which orient perpendicular to the polarization plane of a vertical beam of light.

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